SURVIVAL AND MOVEMENTS OF THE FLATWORM, STYLOCHUS ELLIPTICUS, IN DIFFERENT SALINITIES AND TEMPERATURES

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The flatworm, *Stylochus cllipticus*, an experimentally-proven predator of oysters (Loosanoff, 1956), is one of the most abundant marine polyclads along the Atlantic and Gulf coasts of the United States (Hyman, 1940) and, consequently, may be one of the most important oyster enemies. A review of the literature offered by Hopkins (1949, 1950) showed that there is little published information regarding its physiological behavior. However, Pearse and Wharton (1938) in their report on the physiology of the related species, *Stylochus inimicus*, from Apalachicola Bay, Florida, included some observations on *Eustylochus meridionalis (Stylochus ellipticus)* from the same area. They found that *S. ellipticus* could survive a slow decrease in salinity from 32 parts per thousand to as low as 2.9 ppt, but died in salinities below 6 ppt if the decrease was abrupt. They also reported that the worms became dormant at temperatures below 7° C. These circumstances suggested that some environmental control of *S. ellipticus* might exist in certain areas in cold climates, especially where low salinity and low temperature coincide.

Since information on the physiological behavior of *S. ellipticus* in the northern part of its range is lacking, our studies were initiated to observe the behavior of adult worms (10 to 18 millimeters long) of Milford Harbor, Connecticut, at different salinities and temperatures, with emphasis on observations at low temperatures and salinities. While the salinity of Long Island Sound proper usually fluctuates within a narrow range of approximately 26 to 28 ppt, oyster beds located in estuaries, salt water ponds and rivers may at times, especially in the early spring, be exposed to water that is almost fresh. The annual range of water temperature in this latitude is from about -1° C. to approximately 25° C.

Effects of salinity

Observations were made on survival of the worms in different salinities and the effect of these salinities on movement of these worms. "Righting time," *i.e.*, the time required for a worm to return to normal position after having been turned ventral side up, was used as a quantitative measure. Righting times were determined frequently at each salinity tested; however, more observations were made at the lower salinities. All observations were made in the laboratory at room temperature (18° C. to 22° C.) in standing water, which was changed twice a week. The low salinities were made by diluting sea water from Milford Harbor (about 27 ppt) with tap water demineralized by a Barnstead BD-2 apparatus.

In the first experiment the effects of an abrupt decrease in salinity on survival and righting time of *S. ellipticus* were investigated. Groups of 10 worms each were transferred directly from Milford Harbor water to enamel pans, arranged in pairs, each containing six liters of water of the following salinities: 25, 22.5, 20, 17.5, 15, 12.5, 10, 7.5, 5 and 2.5 ppt and fresh water. Two groups, each containing 10 worms placed in undiluted water from Milford Harbor, served as controls.

Worms transferred directly to salinities as low as 7.5 ppt survived this abrupt change. Those transferred to salinities of 10 ppt showed no distress at any time,



FIGURE 1. Average righting time of *Stylochus ellipticus* from Milford Harbor, Conn. in different salinities at room temperature (18°–22° C.).

while in a salinity of 7.5 ppt some worms, during the first two days, lost color, secreted slightly more mucus than is normal and were sluggish. The worms transferred to 5 ppt were similarly affected, but for a longer time. Four of these died during the first week after transfer, but the survivors eventually ceased to show symptoms of distress and assumed a normal appearance. All of the worms transferred directly to 2.5 ppt and fresh water died within a few hours. However, when some that had become acclimated to a salinity of 5 ppt were transferred to 2.5 ppt, they showed no signs of distress and remained alive and active.

After all symptoms of distress had disappeared in the low salinities, righting times were determined for worms of all groups. The average righting time ranged from 10 to 13 seconds in all salinities, except 5 ppt and 2.5 ppt. In 5 ppt it was 22 seconds and increased to 37 seconds in 2.5 ppt (Fig. 1).

In a second experiment, survival of worms in salinities below 5 ppt was determined after they had been conditioned at intermediate salinities. Three groups of 100 worms each were conditioned for two weeks; one group, at a salinity of 15 ppt;



FIGURE 2. Average righting time of *Stylochus ellipticus* from Milford Harbor, Conn., at different temperatures in a salinity of about 27 ppt.

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FIGURE. 3. Average locomotion rate of *Stylochus ellipticus* from Milford Harbor, Conn., at different temperatures in a salinity of about 27 ppt.

another group, at 10 ppt; and the third, at 5 ppt. Early in the conditioning period 16 of the worms in 5 ppt died, but the remainder of this group became acclimated. None of the worms died or showed distress in either 15 ppt or 10 ppt. At the end of the two weeks of conditioning, groups of ten worms from each conditioning salinity were placed in duplicate enamel pans, each containing six liters of standing water of the following salinities: 2.5, 1.5 and 0.5 ppt and fresh water. Two groups of ten worms each, placed in undiluted Milford Harbor water, served as controls.

Conditioning at intermediate dilutions had little effect on the ability of these worms to survive in low salinities. By the end of the fourth day all the worms conditioned for two weeks at 15 ppt and then transferred to salinities of 2.5 ppt and lower had died. Worms conditioned at 10 ppt had died by the end of the seventh day after transfer. Those conditioned at 5 ppt, and transferred to 1.5 ppt and 0.5 ppt and fresh water, also were dead by the end of the seventh day. Twelve of the 20 worms conditioned at 5 ppt and transferred to 2.5 ppt died by the end of the seventh day, but the remaining eight were alive and healthy when the experiment ended. Only one of 20 worms in the control pans died during the experiment.



FIGURE 4. Average locomotion rates of *Stylochus ellipticus* from Milford Harbor, Conn., and from Apalachicola Bay, Florida at similar water temperatures.

Apparently, the lowest salinity in which *S. ellipticus* of Milford Harbor can survive, even with prior conditioning at an intermediate salinity, is about 2.5 ppt.

Effects of temperature

The effects of temperature on righting time and "locomotion rate," *i.e.*, the rate of forward movement, of *S. ellipticus* were observed in running sea water at 30°, 25°, 20°, 17.5°, 15°, 12.5°, 10° and 7.5° C. The water temperatures were maintained within \pm 1° C. of the desired levels by mixing, in different proportions, heated and unheated Milford Harbor water.

Three groups of ten worms each were allowed to adjust to a selected temperature for three days. Within the next week three separate observations were made on righting time and locomotion rate of all of the worms. At the end of this time the water temperature was changed, the worms were allowed to adjust to the new temperature, and a new series of observations was made. Locomotion rate was determined by noting the length of time needed for each worm to move 40 mm., without stopping, in a straight line.

The average righting time at 20°, 25° and 30° C. was fairly constant at 7 to 9 seconds, but increased to 17 seconds at 17.5° C. and remained at approximately this level at temperatures down to and including 12.5° C. At 10° C. righting time increased to 30 seconds and rose sharply to 116 seconds at 7.5° C. (Fig. 2).

The average locomotion rate varied somewhat erratically at different temperatures, but tended to decrease with decreasing temperature. From a rate of over 50 millimeters per minute at 20° C. and higher it dropped to approximately 10 mm./min. at 7.5° C. (Fig. 3). A comparison of average locomotion rates observed in this experiment with similar data reported by Pearse and Wharton (1938) for *S. ellipticus* from Apalachicola Bay showed that, at the same temperatures locomotion rates of Milford Harbor worms were consistently higher than those of Florida worms (Fig. 4). This difference may indicate the existence of physiological races within this species of flatworm.



FIGURE 5. Average righting time of *Stylochus ellipticus* from Milford Harbor, Conn. at different salinities and temperatures.

Combined effects of temperature and salinity

The combined effects of temperature and salinity on survival and movement of *S. ellipticus* were also studied. Again, righting time was used as a criterion of movement. Duplicate groups of five worms each were held in polyethylene boxes in three liters of standing water of different salinities. These boxes were kept in a water bath where the desired temperatures were maintained.

The procedure used to study the combined effects of temperature and salinity on the righting times of worms was similar to that used in observing the effect of temperature alone, *i.e.*, worms in all salinities were held at the same temperature until their righting times had been determined, then the water temperature was changed and the observations repeated. Using this procedure righting times were obtained in Milford Harbor water at a salinity of about 27 ppt and also in salinities of 15, 10, 7.5 and 5 ppt at temperatures of 20°, 15°, 10° and 7.5° C. Worms tested at 5 ppt were conditioned to this salinity prior to starting the experiment.

Although worms showed some movement in all combinations of temperature and salinity tested, the effects of low temperature and salinity in slowing down their movements became more apparent as the two factors were progressively lowered, and the depressing effects of each factor on their movement tended to reinforce one another (Fig. 5). For example, at 20° C. worms in all salinities from Milford Harbor water to that of 7.5 ppt had approximately the same average righting time, ranging from 11 to 15 seconds, but worms in 5 ppt required an average of 21 seconds to turn over, reflecting primarily the adverse effect of low salinity.

At 15° C. the average righting times of worms in 7.5 ppt and higher salinities were again about equal, but had increased slightly to approximately 18 seconds, due to the effect of the lower temperature. In 5 ppt, however, righting time increased more sharply than expected. For example, at normal salinity a decrease in temperature to 15° C. had no appreciable effect on righting time, and at room temperature lowering of the salinity to 5 ppt had only increased righting time to 22 seconds, but when the temperature was reduced to 15° C. and the salinity to 5 ppt, simultaneously, righting time increased to 79 seconds. The combined effect was, therefore, greater than could have been predicted from the effects of these factors when studied separately.

At 10° C. worms in all salinities were affected by lowered temperature, while those in salinities of 5, 7.5 and 10 ppt showed the exaggerated effect of a combination of low temperature and salinity. For example, in Milford Harbor water and in 15 ppt the average righting time was 32 and 35 seconds, respectively, while at 10 ppt it was 48 seconds, 54 seconds at 7.5 ppt and 103 seconds at 5 ppt.

At a temperature of 7.5° C. the worms in all salinities had slowed their movements still further, but the same pattern of increase in righting times was noted.

In many areas where oyster cultivation is carried on, pronounced variations occur in either temperature or salinity or both. If the rate of predation of flatworms on oysters is closely related to the worms' ability to move about, our studies indicate that predation by these worms must vary considerably at different seasons of the year, especially in a cold climate. Even though their predatory activities may be curtailed by low temperature or low salinity, their ability to survive these adverse conditions makes them a serious threat wherever oyster culture is practiced. We wish to express our appreciation to Dr. V. L. Loosanoff for suggesting this problem and for his critical review of the manuscript. We also wish to thank Mr. Harry C. Davis for his helpful suggestions in the preparation of this paper. Mr. Manton Botsford for the illustrations and Miss Rita Riccio for her careful editing.

SUMMARY

1. Stylochus ellipticus from Milford Harbor, Connecticut, survived abrupt transfer from a salinity of about 27 ppt to salinities as low as 7.5 ppt at room temperature. Those transferred directly to 5 ppt suffered a mortality of 20% but all worms died when placed directly in 2.5 ppt and fresh water. However, worms that acclimated to 5 ppt survived subsequent transfer to 2.5 ppt.

2. Righting time of *S. ellipticus* at room temperature remained constant at 12 to 15 seconds in salinities ranging from about 27 ppt to 7.5 ppt but increased to 22 seconds in 5 ppt and to 37 seconds in 2.5 ppt.

3. Righting time of S. *ellipticus* in a salinity of about 27 ppt was approximately 8 seconds at temperatures of 20°, 25° and 30° C., 16 seconds at 12.5°, 15° and 17.5° C., 30 seconds at 10° C. and 116 seconds at 7.5° C.

4. Locomotion rate of *S. ellipticus* varied erratically with temperature but generally decreased with temperature decreases below 20° C. It exceeded 50 millimeters per minute at temperatures of 20° C. and higher but decreased to 10 mm./min. at 7.5° C.

5. At the same temperatures *S. ellipticus* from Milford Harbor moved faster than reported for the same species from Apalachicola Bay, Florida. This observation suggests that there may be physiological races within this species of flatworm.

6. When temperature and salinity were lowered simultaneously righting time of *S. ellipticus* was frequently longer than the combined righting times obtained when the two factors were observed separately.

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